Chrysopidae family and flower strips as habitat management practices for the control of cabbage insect pests

Guldögonsländor och blomsterremsor som verktyg för bekämpning

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Sammanfattning

Remsor med blommande växter i odlade grödor är en ekologisk strategi som allt oftare kommer till användning för att återställa biodiversiteten i odlingslandskapet. Denna strategi bidrar till att skapa skydd för de naturliga fienderna, ge dem övervintringsplatser, alternativa byten och födoresurser.

Vi har utvärderat effekten av insådda blomsterremsor utformade för att locka till sig och gynna naturliga fiender som angriper skadeinsekter på kål (*Brassica oleracea*), och då speciellt inriktad på guldögonsländor (Chrysopidae). Kålgrödor med närliggande blomsterremsor jämfördes med kontrollrutor utan blomsterremsor. Blomsterremsorna innehöll sex olika växtarter (*Borago officinalis* L., *Coriandrum sativum* L., *Fagopyrum esculentum* Moench, *Foeniculum vulgare* Mill., *Helianthus annuus* L. och *Phacelia tanacetifolia* Benth.) som dokumenterats ha blommor med positiva effekter på överlevnad, reproduktion, utveckling och attraktion av guldögonsländor.

Förekomsten av de fem olika skadegörare som undersöktes i studien (*Mamestra brassicae* L., *Plutella xylostella* L., *Pieris brassicae* L., *P. rapae* L. och *Brevicoryne brassicae* L.) var signifikant högre i kontrolleden (9.32 ± 3.11) jämfört med leden intill blomsterremsorna (4.06 ± 0.89). I blomsterremsorna ökade förekomsten av parasitoider och predatorer signifikant med ökad blomningsintensitet av de ingående blomväxterna. *Chrysoperla carnea* (Stephens, 1836) sense Henry och *C. lucasina* (Lacroix, 1912) representerade 90,8% av den totala mängden fångade vuxna guldögonsländor i blomsterremsorna. Under både laboratorie- och fältförhållanden föredrog guldögonsländor pollen från *P. tanacetifolia* och *C. sativum* och konsumtionen av pollen var signifikant högre för honor jämfört med hanar.

Sammanfattningsvis, blomsterremsor kan ha en positiv effekt på den biologiska bekämpningen av skadegörare i kålgrödor. Genom att etablera blomsterremsor skulle dessa kunna vara ett alternativ till användningen av växtskyddsmedel genom att förbättra betingelserna för lokalt förekommande naturliga fiender med förutsättningar att kontrollera de allvarligaste skadegörarna i kålgrödor. *P. tanacetifolia* pekas ut speciellt som en mycket lämplig växt att uppföröka förekomsten av *C. carnea* i fält. Förstärkning av mångfalden av naturliga fiender med hjälp av blommande växter, som har observerats för guldögonsländor, kan förbättra de naturliga fiendernas resiliens och tillhandhålla ytterligare ekosytemtjänster som pollinering. Som en allmän rekommendation vill vi uppmuntra forskare, odlare och rådgivare att introducera användningen av blomsteremsor i integrerade växtskyddsprogram som en lovande metod att förstärka den funktionella biodiversiteten för biologisk bekämpning.

Abstract

Flowering plant species within a cultivated field are increasingly implemented as an eco-scheme to re-establish functional agrobiodiversity providing shelter, overwintering sites, and alternative prey and food resources to natural enemies (predators and parasitoids).

We evaluated the effect of sown flower strips designed to attract and support natural enemies of *Brassica oleracea* pests, with a special emphasis on green lacewings (Chyrsopidae). Brassica plant blocks with adjacent flower strips were compared with control plant blocks. Flower strips were comprised by six plant species (*Borago officinalis* L., *Coriandrum sativum* L., *Fagopyrum esculentum* Moench, *Foeniculum vulgare* Mill., *Helianthus annuus* L. and *Phacelia tanacetifolia* Benth.) which provides floral resources with demonstrated positive effects on survival, reproduction, development and recruitment of lacewings.

The abundance of the five pest species considered in this study (*Mamestra brassicae*, *Plutella xylostella*, *Pieris brassicae*, *P. rapae* and *Brevicoryne brassicae*) was significantly higher in control blocks (9.32 ± 3.11) than in blocks next to the flower strips (4.06 ± 0.89). In the flower strips, parasitoid and predator abundances increased significantly with increasing blooming rates of the selected flowering species. *Chrysoperla carnea* (Stephens, 1836) *sensu* Henry and *Chrysoperla lucasina* (Lacroix, 1912) represented 90.8% of the total green lacewing adults collected from flower strips. In laboratory and field conditions, green lacewings clearly preferred pollen from *P. tanacetifolia* and *C. sativum*, being the consumption of pollen grains significantly higher for females compared to male specimens.

In conclusion, flower strips may have a positive effect on biological pest control in brassica crops. Indeed, the use of flower strips may be an alternative to pesticide applications, creating a more favourable environment for locally occurring natural enemies that have the potential to control key brassica pests. *P. tanacetifolia* is pointed out as a particularly suitable plant to increase the presence of *C. carnea* in agricultural fields. Enhancing natural enemy biodiversity by using flower resources, as observed for green lacewings, can increase the resilience of natural pest control and provide additional ecosystem services such as pollination. As a general recommendation, we encourage researchers, farmers and practitioners to include the establishment of flower strips in their integrated pest management programs as a promising tool to protect functional biodiversity for biocontrol.

1. Introduction

The exacerbated intensification of agriculture has had negative repercussion on the wide environment, leading to landscape simplification, spill over of agrochemicals in soil and water and increasing loss of biodiversity (Conway, 2000; Tilman et al., 2002). Farmers are experiencing major losses of essential ecosystem services to crops, such as pollination, water and fertility regeneration, nutrient recycling and biological control (Steffen et al., 2015). Ecological intensification has been proposed as a shift towards a more ecosystem-based agriculture (Tittonell, 2014). The re-establishment of functional agrobiodiversity in the cultivated landscape though habitat management is among the new trajectories included in this paradigm shift. Functional agrobiodiversity contemplates the introduction of non-crop vegetation with the aim to provide shelter, overwintering sites, and alternative prey and food resources to natural enemies (predators and parasitoids) (Gurr et al., 2017; Landis et al., 2000). Among the different ways of adding plant diversity, strips of flowering species within a cultivated field gained in the last two decades a growing interest by researchers, farmers and practitioners (Haaland and Gyllin, 2011; Penvern et al., 2019). Although with a variable effect on the target crop, annual and perennial flower strips have been shown to enhance beneficial arthropod diversity and, as a consequence, biological control of insect pests (Cahenzli et al., 2019; Fiedler et al., 2008; Tschumi et al., 2016). However, several factors may influence the success of the strategy, and therefore system-specific evaluations are required before implementation (Tscharntke et al., 2016). Parameters such as time of flowering during the season, synchronization with natural enemies and pest occurrence, production of nectar and pollen, and competition for resources with the main crop should be carefully investigated in advance. Furthermore, flower strips should not be able to attract and host arthropods potentially harmful to the main crop, or favour intraguild predation between beneficial arthropods.

Brassica are important arable crops in Europe attacked by a range of herbivores, including insects, nematodes, slugs and birds (Alford et al., 2003). The most important Brassica vegetables in Sweden are cauliflower, white cabbage and broccoli (SCB, 2018). Several key Brassica insect pests have developed resistance to certain widely used pesticides making their control increasingly difficult (Heimbach and Müller, 2013). One alternative to the use of pesticides is to create a more favourable environment for locally occurring natural enemies that have the potential to control key pests (Balmer et al., 2014; Nilsson et al., 2016). For instance, a flower mixture and single plant species were evaluated to improve natural pest control of lepidopteran pests, with positive effects such as increased parasitism rates on *P. xylostella* and *P. rapae*, and predation on *M. brassicae*. However, these positive effects have been found only in part of the studies (Lee and Heimpel, 2005; Pfiffner et al., 2009; Winkler et al., 2009; Zhao et al., 1992).

Green lacewings are one of the well-studied groups of natural enemies due to their importance as allies in crop protection, being abundant in field crops and widespread around the world (Pappas et al., 2011). The *Chrysoperla carnea*-group, which includes the most dominant green lacewing species in agroecosystems, is comprised at least by 21 sibling species extremely similar in morphology (Price et al., 2015). Three or four species of the group may coexist, even on the same tree (Alcalá Herrera et al., 2019; Henry et al., 2013). Agroecological studies on green lacewings rarely break down the *carnea*-group into its species assemblage, missing essential insight into the ecological differences among species. Green lacewings prey, mainly in their larval stage, on soft-bodied insects such as aphids, thrips, mites, moths, and lepidopteran eggs (Principi and Canard, 1984). Most adults have glycol-palynophagous habits, with a diet based on pollen, nectar and honeydew (Canard, 2001; Stelzl, 1992, 1991; Villa et al., 2017; Villenave et al., 2006). The species of the *carnea*-group are opportunistic feeders which are attracted to large patches of flowering plants, and pollen is consumed according to its availability by time of blooming and abundance (Alcalá Herrera et al., 2020; Villenave et al., 2006). Villenave et al. (2006) suggested that bordering vegetation is the reservoir habitats and resting places for most chrysopids.

Some studies assessing the effect of cover crops and flower strips on natural enemies found no evidence of increased lacewing activity during the cropping season (Bone et al., 2009; Smith et al., 1996). Conversely, other studies have observed that flowering plants in apple, citrus and olive orchards, as well as sown flower strips sown adjacent to cotton and cabbage, increased lacewings populations and recruitment into the crop (Burleigh et al., 1973; Fye and Carranza, 1972; Porcel et al., 2017; Ridgway and Murphy, 1984; Silva et al., 2010; Wyss, 1995). There are several factors to consider in order to increase and maintain naturally occurring chrysopid populations through habitat management (Ridgway and Murphy, 1984). First, the availability and suitability of plant species, i.e. flowers with big corollas (accessible pollen) and well exposed nectaries (Nave, 2016; Van Rijn and Wackers, 2016; Villenave et al., 2005). Second, habitat suitability as reproduction site, linked with the presence of preimaginal-stages (Bianchi et al., 2013). Finally, the knowledge on local population dynamics of both chrysopids and pest target species may contribute to an effective design of habitat management strategies (Landis et al., 2000; Ridgway and Murphy, 1984).

Thus, the overall goal of this study was to evaluate whether selected native flowering plant species arranged in strips can be used to attract and support natural enemies with a special emphasis on green lacewings. In addition, we estimated their suitability as a tool to enhance biological pest control of the lepidopterans *M. brassicae, P. xylostella, P. brassicae, P. rapae* and the aphid *Brevicoryne brassicae* L. by green lacewings in organic *Brassica oleracea* crops. We hypothesised that adult lacewings would be attracted and use flower resources as pollen and nectar and locate suitable oviposition sites in adjacent cabbage crops, where their larvae will prey on immature stages of the most important cabbage pests. The specific objectives of this study were: (i) to evaluate the impact of flower resources on pests and predators in brassica crops, (ii) to identify the arthropod community, especially green lacewings, associated to the plant species in the flower strips, (iii) to examine the pollen consumed by lacewing adults collected from the flower strips and (iv) to establish their pollen feeding preferences in the laboratory.

2. Material and methods

2.1 Experimental design

The field experiment was conducted between May and September 2017. Three commercial farms were selected in the county of Scania in southern Sweden (Figure 1), an area dominated by agriculture consisting of arable land and meadows. Among the most important crops are predominantly rye, barley, oats, wheat, sugar beets and potatoes. All three farms were organically certified under UE regulation by KRAV Sweden. Each farm had a surface planted with cabbage (*Brassica oleracea*), with a range of three to seven varieties per farm. In each farm standardized 1.80 metres wide and between 20 to 180 m long flower strips were established between May and June in 2017 (Figure 1).

In the three farms, the cabbage plants were sown in two treatments: (i) adjacent to flower strips (flower strip blocks) and (ii) with no adjacent flower strips (control blocks) (Figure 2). A diagonal line of eighteen cabbage plants, randomly selected and marked the first time, was regarded as a block. The number of blocks per farm depended on the cabbage crop surface available by the grower´s and to maximize differences between treatments. In farm 1 there were 20 blocks (5 control blocks and 15 flower strip blocks), 9 blocks in farm 2 (2 control blocks and 7 flower strip blocks) and 6 blocks in farm 3 (3 control blocks and 3 flower strip blocks) (Figure 1). Blocks were sampled repeatedly on six to eight occasions in each farm at least once a week from June to September. The minimum distance between blocks and treatments were 5 and 25 m, respectively. The minimum distance between control blocks and rows was 17 m (Figure 1).

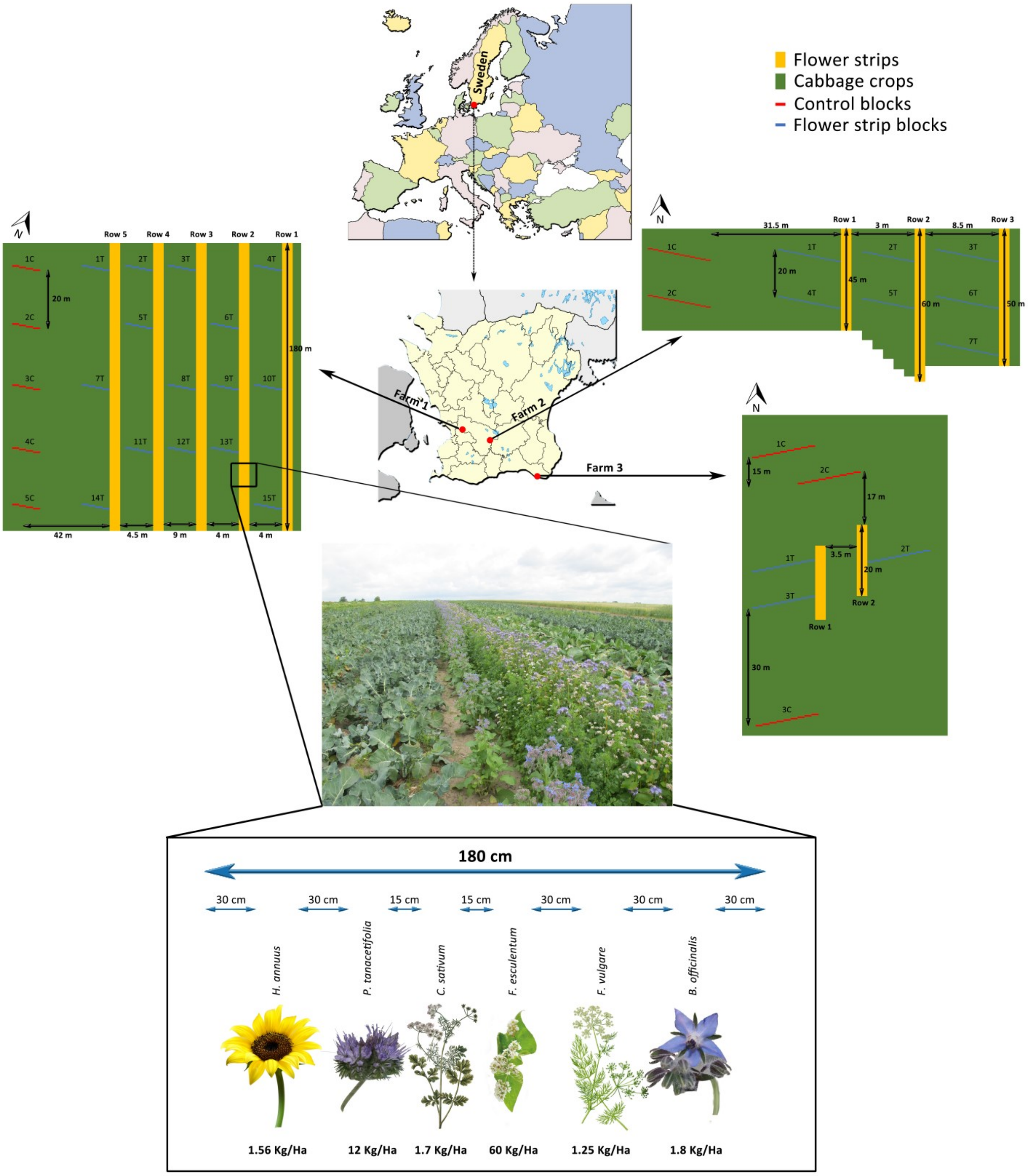

Figure 1. Diagram showing farms locations, flower strips distribution, plant species distribution within the flower strips and seed rate per plant species.

The flower strips were comprised by six different plant species (Table 1 and Figure 1). These species were chosen based on the two following criteria: i) floral resources with demonstrated positive effects on survival, reproduction, development and population dynamics of lacewings (Gonzalez et al., 2016; Robinson et al., 2008;

Tschumi et al., 2016; Van Rijn, 2012; Villa et al., 2016; Villenave et al., 2006, 2005) and ii) the locally adapted ecotype plant species are present throughout Sweden and around the world.

Table 1. Plants sown in the flower strips.

Scientific name	Common name	Family	Seed quantity (kg/ha)
Borago officinalis L.	Starflower	*Boraginaceae*	1.8
Coriandrum sativum L.	Coriander	*Apiaceae*	1.7
Fagopyrum esculentum Moench	Buckwheat	*Polygonaceae*	60
Foeniculum vulgare Mill.	Fennel	*Apiaceae*	1.25
Helianthus annuus L.	Sunflower	*Asteraceae*	1.56
Phacelia tanacetifolia Benth.	Lacy phacelia	*Boraginaceae*	12

Seeds of the plant species were produced under organic conditions and supplied by a company specialised in locally adapted ecotypes (Lindbloms Frö, Sweden). Plant species were sown by a hand seeder in single species rows with plants within the same flower strip sown at the same time. The row to row and plant to plant spacing was determined by the plant spacing specification (Figure 1). However, we synchronized the sowing time of flower strips to growers planting schedules (Figure 1). Weeds were manually managed, and flower strips were irrigated at the same time as the cabbage crops. No pesticide treatments, mowing or fertilization were conducted in the flower strip.

Flower bloom was estimated as the percentage of open flowers (0-100%) for each plant species in each row and farm at least once a week from June to September, with a total of between six to nine times per farm.

2.2 Pests and predator presence in brassica crops

All leaves and the stem of the cabbage plants in flower strip and control blocks were closely inspected to count and record the presence and abundance of the lepidopterans *M. brassicae*, *P. xylostella*, *P. brassicae*, *P. rapae* and the aphid *B. brassicae*, as well as the generalist predators Chrysopidae, Coccinellidae and Syrphidae.

2.3 Arthropod collection in flower strips

Arthropod collection started around the 15th July, approximately seven weeks after sowing the flower strips and once the flowers started to bloom and finished by the 1st September. Flower strips were weekly vacuumed for 40 seconds with a Stihl® SH 85C aspirator (Stihl AG & Co, Waiblingen, Germany) from July to September. The number of samples per farm ranged from eight to 30 in each sampling occasion. Suction samples were kept in cold for transportation and stored in a freezer at -20°C until identification.

The arthropods collected were identified to family or genus level when possible using standard taxonomic keys. We pooled the individuals collected into two groups of natural enemies: predators (Chrysopidae, Coccinellidae, Cantharidae, Anthocoridae, *Deraeocoris sp.* and Nabidae) and parasitoids (Ceraphronidae, Megaspilidae, Aphelinidae, Encyrtidae, Eulophidae, Mymaridae, Pteromalidae, Torymidae, Cynipidae, Figitidae, Braconidae, Ichneumonidae, Platygastridae, Diapriidae, Proctotrupidae).

Chrysopidae larvae were determined under stereomicroscope to genus level based on larval cephalic chaetotaxy (Monserrat, 2016). Chrysopidae adults were sexed and identified to species level following Plant (2013) and Aspöck et al. (1980). Cryptic species of the *carnea*-group, excluding *Chrysoperla lucasina* (Lacroix, 1912), were identified to species level as in Henry et al. (2002) and Chapman et al. (2006), by measuring the basal dilation of the metatarsal claw and the genital lip and chin of the male abdomen and comparing the values to those reported by Henry et al. (2002).

2.4 Pollen-foraging preference assay

A laboratory assay was performed in August 2017 to assess the pollen foraging preference of the common green lacewing to the different plants used in the field experiment. The species *Chrysoperla carnea* (Stephens 1836), the most recorded in the field trial, was used for the laboratory experiment. Adults were obtained from a stock colony established for the trial with individuals provided by Sautter & Stepper GmbH (Ammerbuch-Altingen, Germany). *C. carnea* adults were fed with a mix of 1:1 v/v honey:pollen and kept in a plastic cage (31 x 21 x 12 cm) inside an incubator under controlled conditions at 25±2ºC, 50–60% of humidity and 16:8 hours L:D photoperiod. Recently laid eggs were harvested and isolated in plastic Petri dishes (5 cm in diameter). After hatching, larvae were fed *ad libitum* with *Ephestia kuehniella* Zeller eggs (Biotop, Livron-sur-Drôme, France) until pupation. Newly emerged adults were sexed and starved for 24 h. An adult female and an adult male of *C. carnea* were placed inside a plastic cage (18 x 18 x 10 cm) with a couple of well-developed flowers with stems arranged in a 25 ml water cup in the following combinations: *P. tanacetifolia* vs *B. officinalis*, *P. tanacetifolia* vs *C. sativum*, *P. tanacetifolia* vs *F. esculentum*, *F. esculentum* vs *B. officinalis*, *F. esculentum* vs *C. sativum* and *C. sativum* vs *B. officinalis*. Flowers were obtained from fresh plant material collected daily from the flower strips and kept in water. Adults were allowed to feed from the flowers for 24 h. Afterwards they were immediately frozen at -20ºC for gut content analysis. Six replicates of all possible combinations were run for a total of 72 *C. carnea* adults assayed (36 females and 36 males).

Gut content analysis was performed to establish *C. carnea* pollen preference in the pollen-foraging trial. We chose the acetolysis technique because it allows digesting proteins, lipids, insect tissues and pollen grain debris, so accurate pollen identification can be carried out easier (Jones, 2014). This methodology has been previously used to study gut in Chrysopidae (Andrade et al., 2017; Medeiros et al., 2010; Resende et al., 2017; Villa et al., 2019). Frozen adults were defrosted at room temperature. Wings, legs and antennae were removed to reduce residues after acetolysis. After removing the legs, each specimen was placed into a 2 ml microcentrifuge tube filled with 96% ethanol and washed and vortexed three times to clean external pollen on the lacewing's body. Then, it was transferred into a new 2 ml microcentrifuge tube with 0.5 ml of glacial acetic acid, vortexed and discarded the glacial acetic acid to eliminate any residual water from the sample. The specimen was smashed with the aid of a clean micropestle to improve the digestion, and 0.5 ml of acetolysis mixture (9:1 glacial acetic acid to sulfuric acid) was added. The microcentrifuge tube was heated (100°C) in a dry heater for eight minutes. Afterward, 0.5 ml of glacial acetic acid was added to stop the acetolysis process, centrifuged for 10 min at 10000 rpm, and the supernatant was discarded. The remaining reagents were cleaned washing the sample three times with one ml of distilled water. The sample was vortexed, centrifuged again (for three minutes at 10000 rpm), and the supernatant was discarded. Finally, the sample was stored in a fume hood at 70ºC for 12 h to evaporated remaining distilled water.

For microscope slides preparation, ten microliters of a mix of 99% glycerine and distilled water (1:1) were added to the microcentrifuge tube, mixed with the sample, placed on the slides, and protected with a glass coverslip sealed with nail varnish.

For pollen examination, three traverses were carried out across the coverslip at the centre of each slide under microscope at 400x (to count) and 1000x magnifications (to identify). The pollen was identified based on its morphological polar and equatorial axes, shape, apertures and exine ornamentation (Punt and Clarke, 1980, Valdés et al., 1987 Faegri, K. and Iversen, 1989). The results obtained were compared with the surrounding vegetation in the farm, the reference pollen collection from fresh flowers obtained from the flower strips, and slides created from specimens feeding from a single known plant species. Pollen grains size varies enormously between plant species (Punt and Clarke, 1980). To document the importance of pollen volume on Chrysopidae diet, we measured the pollen abundance in two different ways: as counts of pollen grains and volume of pollen grains, as in a previous study on bees (Buchmann and O'Rourke, 1991).

Once the Chrysopidae adults from sections 2.3 were identified to species level, their gut contents were examined following the methodology described to establish their pollen preferences in natural conditions.

2.5 Statistical analysis

We analysed the data using R v.3.6.3 (R Development Core Team, 2017) and R Studio v.1.1.456 (RStudio Team, 2016), with packages *glmmTMB*, (Brooks et al., 2017), *Matrix* (Bates and Maechler, 2019), *lme4* (Bates et al.,

2014) and *vegan* (Oksanen et al., 2018). For each model, residuals plots were examined for model validation using package *DHARMa* (Hartig, 2018). Regarding data from sections 2.2, 2.3 and 2.4, we checked fixed factors for significance with Wald tests using the *car* package (Fox and Weisberg, 2019) and multiple comparison between levels of the fixed factor were tested using Tukey's test with package *emmeans* (Lenth, 2020, 2016).

To investigate the effect of farm and flower bloom on the number of predators and parasitoids, suction sampling data were analysed using generalized linear mixed models (GLMM). In each model, sampling date and flower strips (row number) were established as random effects to account for repeated measures on the same row at different times and population dynamics, respectively. Models were corrected for overdispersion by using a negative binomial (NB) distribution or adding a quasi-Poisson structure. We used a non-metric multidimensional scaling (NMDS) based on the Bray-Curtis distance to represent graphically the level of association between the different predator and parasitoid groups with farm and flower bloom of each plant species. For each NMDS, stress was checked with the aid of the goodness plot and Shepard stress test. A permutational multivariate analysis of variance (PERMANOVA) with Bray-Curtis distance and 999 permutations was performed to test if the predator and parasitoid communities differed between farms and were affected by flower bloom of the different plant species.

The visual sampling data revealed a low number of pest and predators in cabbage crop. Thus, we used hurdle generalized linear mix models (hurdle GLMM) with a NB and Poisson error distribution to investigate the effect of floral attractiveness, farm and treatment (control block and block next to flower strip) on number of pests and predators. In each model, sampling date was set as random effect to correct for repeated measures over time.

To analyse pollen-foraging preferences, the number and volume of pollen grains from the dual-choice assay were analysed using GLMMs with a NB distribution, while the volume of pollen grains were analysed using LMMs. Models included lacewing sex as fixed factor covariable and specimen as random effect because of the paired-data recorded on the same combination of plant species. Furthermore, separate NB GLMM and LMM were performed for the whole dataset to establish the differences between sexes in number and volume of pollen grains consumed respectively. In each model, specimen and plant species were established as random effects.

We used a GLMM with NB distribution to test number of pollen grains, and a LMM to test the volume of pollen grains identified in the gut content of Chrysopidae species collected by suction sampling (section 2.3). Models included sex, plant species (*B. officinalis*, *C. sativum*, *F. esculentum* and/or *P. tanacetifolia*), chrysopid species (*Chrysopa commata* data was excluded due to the low number of adults collected) and flower bloom as fixed effects. The interaction term between plant species and flower bloom was included to explore the effect of bloom at different times on pollen utilization by the lacewings. Both models included farm as random effect.

3. Results

3.1 Impact of flower strips on predators and pest in brassica fields

The number of predators and pests recorded by visual inspection in brassica plants throughout the season was 0.23 ± 0.04 (mean ± SE) and 5.68 ± 1.15 individuals per block, respectively (Figure 2). The predator population showed two peaks of abundance in the middle of July and in the beginning of August (Figure 2A). The pest population was relatively low as compared to predators during almost all the sampling period, with a sharp increase at the end of August (Figure 2B). Among the five pest species considered in this study, *P. brassicae* and *P. rapae* were the most abundant (Figure 2B). Regarding predators, Coccinellidae, Syrphidae and Chrysopidae were the dominant groups. We observed a drastic reduction in pest abundance in flower strip blocks (4.06 ± 0.89) compared with control blocks (9.32 ± 3.11) (Hurdle model, $\chi^2 = 9.6$, d.f. = 1, $P < 0.01$), farm (Hurdle model, $\chi^2 = 15.1$, d.f. = 2, $P < 0.001$) and flower bloom variables (Hurdle model, $\chi^2 = 6.4$, d.f. = 1, $P = 0.012$). The abundance of predators in the crop was not influenced by any of the three variables tested (Hurdle model, $P > 0.050$)

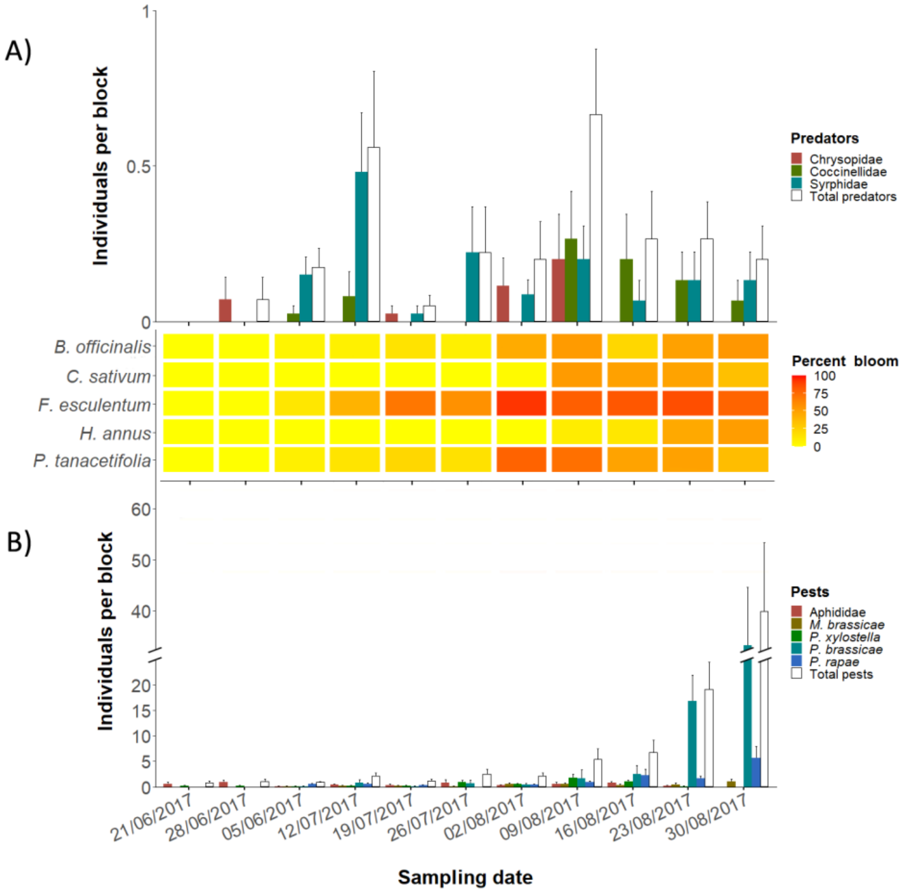

Figure 2. Number of A) predators and B) pests (mean ± SE) recorded in each sampling date by visual inspection of brassica plants. The heatmap represents the percent bloom of each plant species in the flower strips.

3.2 Arthropod collection in flower strips

A total of 38,081 arthropods were collected from the flower strips using the vacuum device. Order Diptera was the most abundant, representing 50.2% of the total, followed by Order Thysanoptera (13.81%) and other Coleoptera (5.27%).

In terms of functional groups, parasitoids represented 17% of the total arthropods with 6,349 individuals, being the most abundant the families Pteromalidae (4.8%), Braconidae (3.61%) and Eulophidae (3.5%), while predators, with only 2.59%, were represented mainly by the groups *Orius sp.* (0.95%), Syrphidae (0.64%), *Anthocoris sp.* (0.46%), Neuroptera (0.33%); Coccinellidae (0.12%), *Deraeocoris sp.* (0.05%), Cantharidae (0.03%) and Nabidae (0.01%). The number of predators increased gradually over the sampling period from the beginning of July to the highest peak of abundance at the end of August

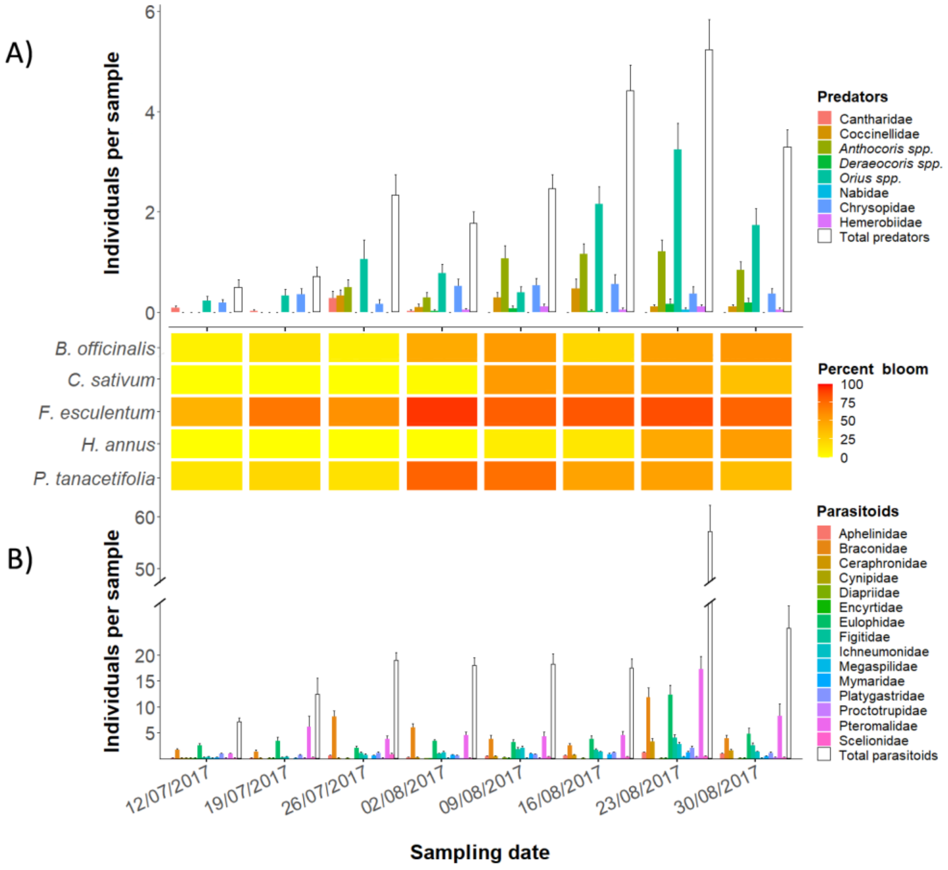

Figure 3. Number of A) predators and B) parasitoids (mean ± SE) in each sampling date collected by suction sampling from the flower strips. The heatmap represents the percent bloom of each plant species in the flower strips.

The same trend was recorded for parasitoids, with abundances increasing from the first sampling date to the highest value recorded at the end of August (Figure 3B). Parasitoid and predator abundances increased significantly with increasing flower bloom in the flower strips (Figure 4).

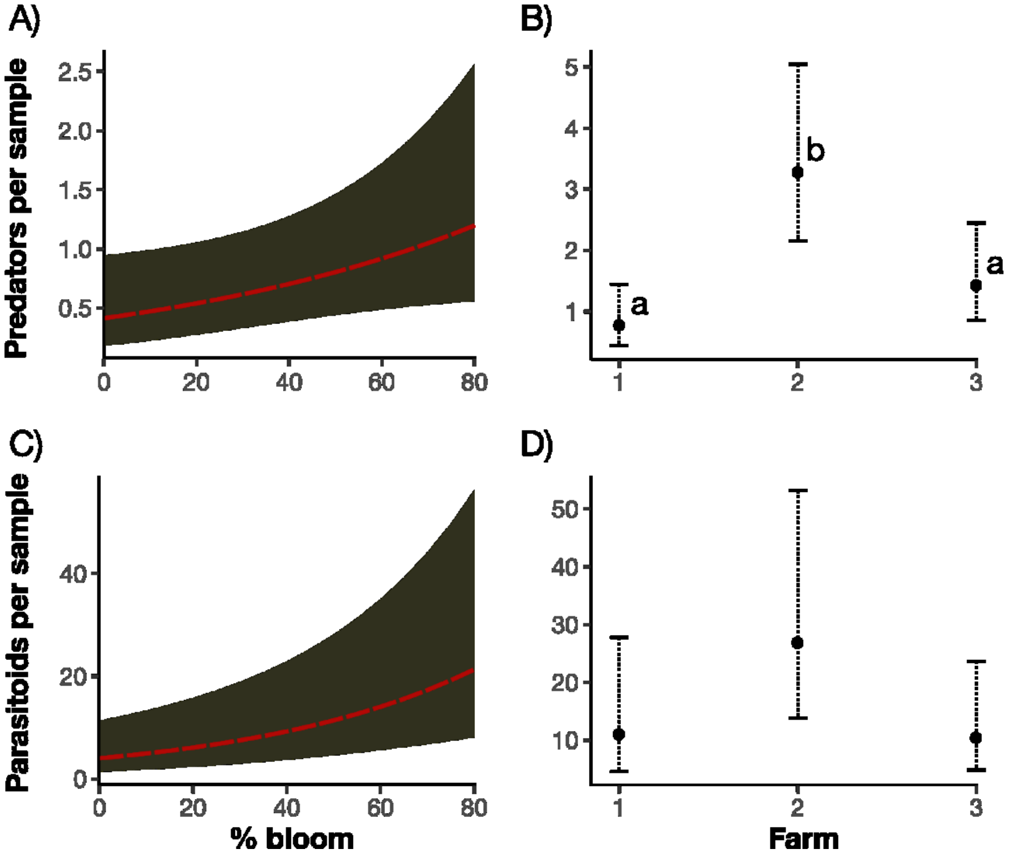

Figure 4. GLMM predicted values (±95% CI) of the relationship between mean percentage of flower bloom in the flower strips and A) predators ($\chi^2 = 3.9$, d.f. = 1, $P = 0.047$) and C) parasitoids ($\chi^2 = 14.2$, d.f. = 1, $P < 0.001$) per aspiration sample, and B) predators ($\chi^2 = 17.9$, d.f. = 2, $P < 0.001$) and C) parasitoids ($\chi^2 = 4.8$, d.f. = 2, $P = 0.092$) per sample in the different farms.

Predatory insect community analysis showed significant differences between farms (PERMANOVA, $R^2 = 0.546$, Pseudo-F = 16.2, d.f. = 2, $P < 0.001$), but no effect of flower bloom (PERMANOVA, $R^2 = 0.040$, Pseudo-F = 2.5, d.f. = 2, $P = 0.076$) on community composition (Figure 5A). Cantharidae was associated with *P. tanacetifolia* bloom, and Coccinellidae, *Orius sp.*, *Anthocoris spp. Deraeocoris spp.* were associated with *B. officinalis*, *F. esculentum*, *C. sativum*, and *H. annus* bloom. The parasitoid community was significantly affected by both farm (PERMANOVA, $R^2 = 0.494$, Pseudo-F = 17.1, d.f. = 2, $P < 0.001$) and flower bloom (PERMANOVA, $R^2 = 0.102$, Pseudo-F = 7.1, d.f. = 2, $P < 0.003$) (Figure 5B). Encyrtidae were associated with *C. sativum* and *H. annus* bloom while Megaspilidae and Torymidae were associated with *B. officinalis* and *F. esculentum* bloom. The rest of parasitoid families are associated with *P. tanacetifolia* (Figure 5B).

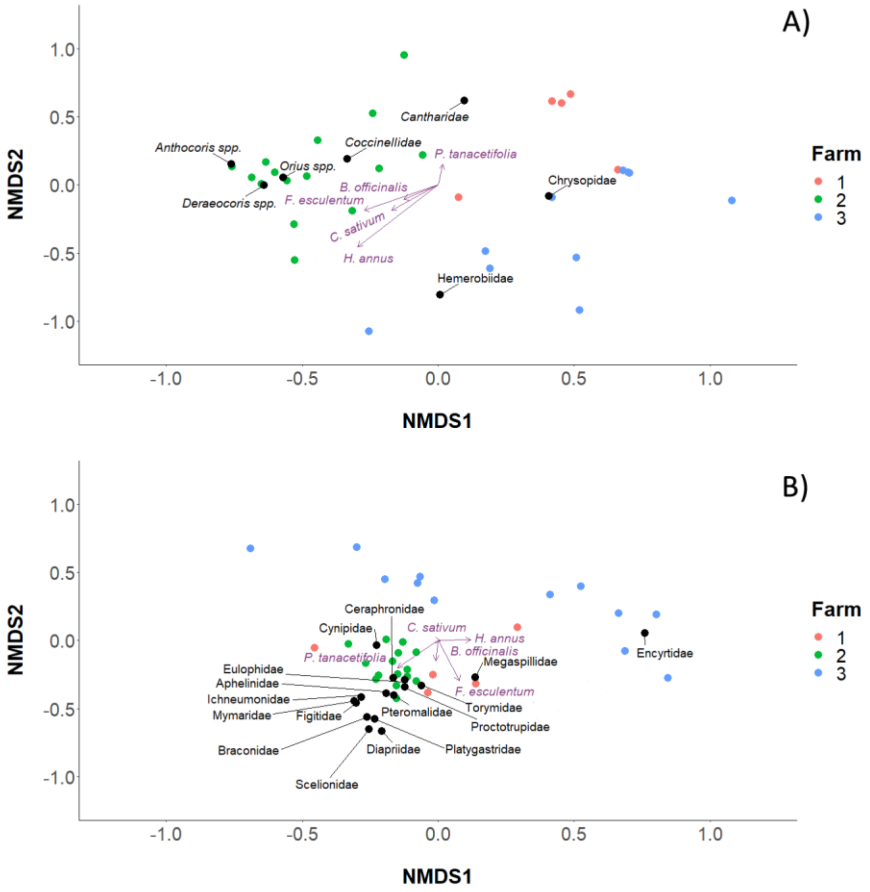

Figure 5. Non-metric multidimensional scaling (NMDS) biplot representing the community composition of A) predators (Stress = 0.156) and B) parasitoids (Stress = 0.143) collected by suction sampling from the flower strips. Coloured points indicate different farms, black points indicate the position of predator and parasitoid groups in the multivariate space. Plant species in relation to the predators and parasitoids are represented by orchid arrows. Farms differed in both predator and parasitoid communities (PERMANOVA, Pseudo-*F* = 16.2*, P* < 0.001*;* Pseudo-*F* = 17.1*, P* < 0.001*;* respectively). The parasitoid community composition was affected by flower bloom of the plant species in the flower strips (PERMANOVA, Pseudo-*F* = 7.1*, P* < 0.003).

3.2 Pollen consumption by Chrysopidae

Chrysoperla carnea individuals consumed significantly more pollen from *P. tanacetifolia* and *C. sativum* as compared to *F. esculentum* and *B. officinalis* (Figure 6A), with no differences between both paired plant species. The overall number of pollen grains consumed was significantly higher for females compared to male specimens (Figure 6B).

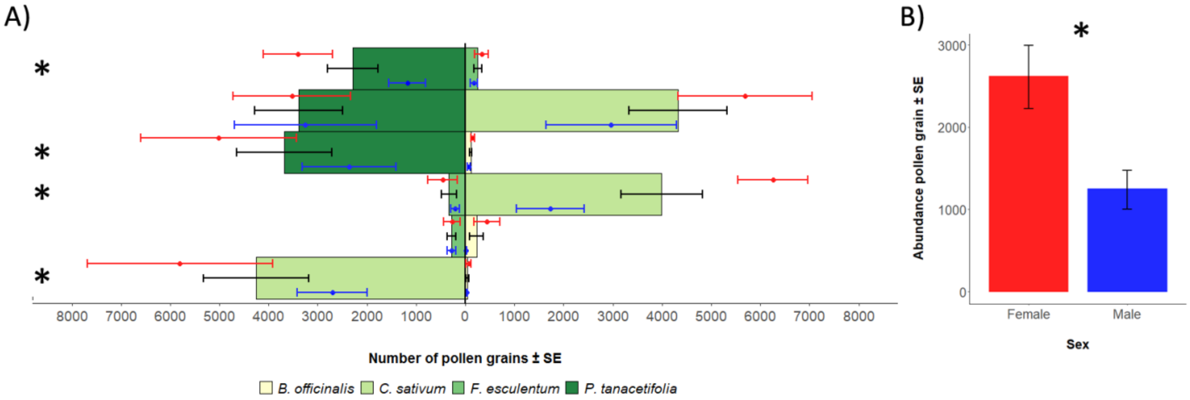

Figure 6. A) Pollen grains (mean ± black SE) consumed by *C. carnea* in each dual choice combination. The red dots and error bars (mean ± SE) represent pollen consumption by females and the blue dots and error bars (mean ± SE) represent pollen consumption by males for each combination.. B) Pollen grains consumed by sex (mean ± black SE) for the whole trial. Differences in pollen quantity consumed between paired plants and between sexes are indicated with an asterisk (GLMM, Wald test, $P < 0.05$).

Regarding the pollen volume consumed, we observed the same results as for the number of pollen grains (Figure 7).

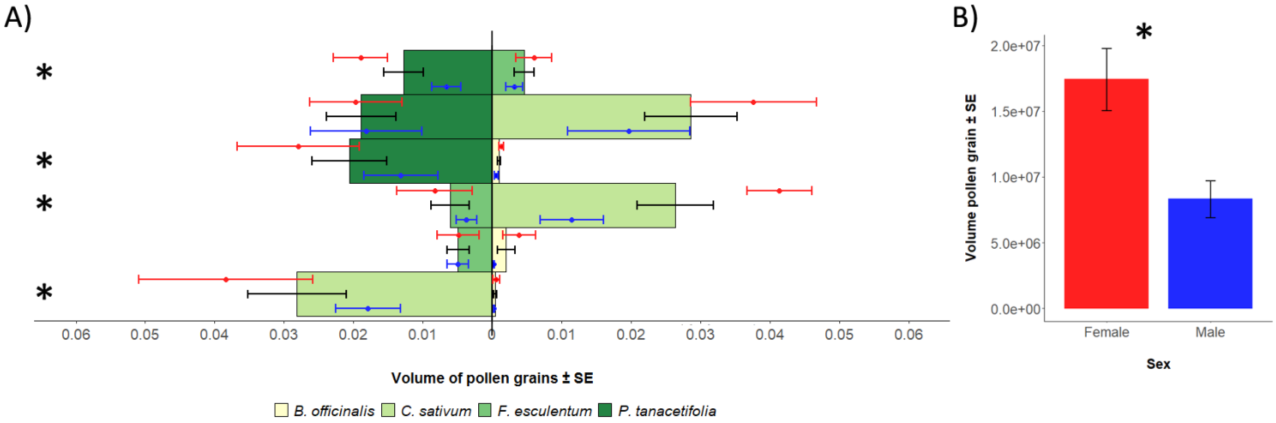

Figure 7. A) Volume of pollen grains (mean ± black SE) consumed by *C. carnea* in each dual choice combination. The red dots and error bars (mean ± SE) represent pollen consumption by females and the blue dots and error bars (mean ± SE) represent pollen consumption by males for each combination. B) Pollen volume consumed by sex (mean ± black SE) for the whole trial. Differences in pollen volume consumed between paired plants and between sexes are indicated with an asterisk (GLMM, Wald test, $P < 0.05$).

A total of 54 adults (35 females and 19 males) and 59 larvae from the Chrysopidae family were captured in the flower strips. Adult chrysopid specimens were identified as 48.2% *Chrysoperla carnea* (Stephens, 1836) *sensu* Henry, 42.6% as *C. lucasina* and 9.3% as *Chrysopa commata* Kis & Újhelyi, 1965 (Table 2). *Chrysoperla pallida* Henry, Brooks, Duelli and Johnson*,* 2002, the other sympatric sibling species from the *carnea*-group in northern

Europe, was not recorded in this study. The 59 larvae collected were identified at genus level as 71.2% *Chrysoperla sp.* and 28.8% as *Chrysopa sp.* Leach, 1815 (Table 2).

Table 2. Taxonomic identification and abundance of chrysopids collected from the flower strips.

Taxonomical identification	Adult		Larvae
	male	female	
Chrysoperla Steinmann, 1964			42
Chrysoperla carnea (Stephens, 1836) sensu Henry	7	19	
Chrysoperla lucasina (Lacroix, 1912)	9	14	
Chrysopa Leach, 1815			17
Chrysopa commata Kis & Újhelyi, 1965	3	2	

A total of 112,319 pollen grains were counted inside the adult chrysopid guts. We observed that adults fed from all the plant species present in the flower strips, but the pollen consumed from *P. tanacetifolia* was considerably higher compared to the rest, both in quantity and in total pollen volume (Figure 8A, Figure 9). A small percentage (5.61%) of the pollen consumed corresponded to other plant species such as the families Asteraceae (1.24%), Brassicaceae (3.32%), Cariophyllaceae (0.59%), Pinaceae (0.001%), Poaceae (0.18%), Polygonaceae (0.03%) and Rosaceae (0.19%). No statistical differences between chrysopid species were detected (Figure 8B). In addition, we observed that chrysopid female adults consumed more pollen than males (Figure 8C).

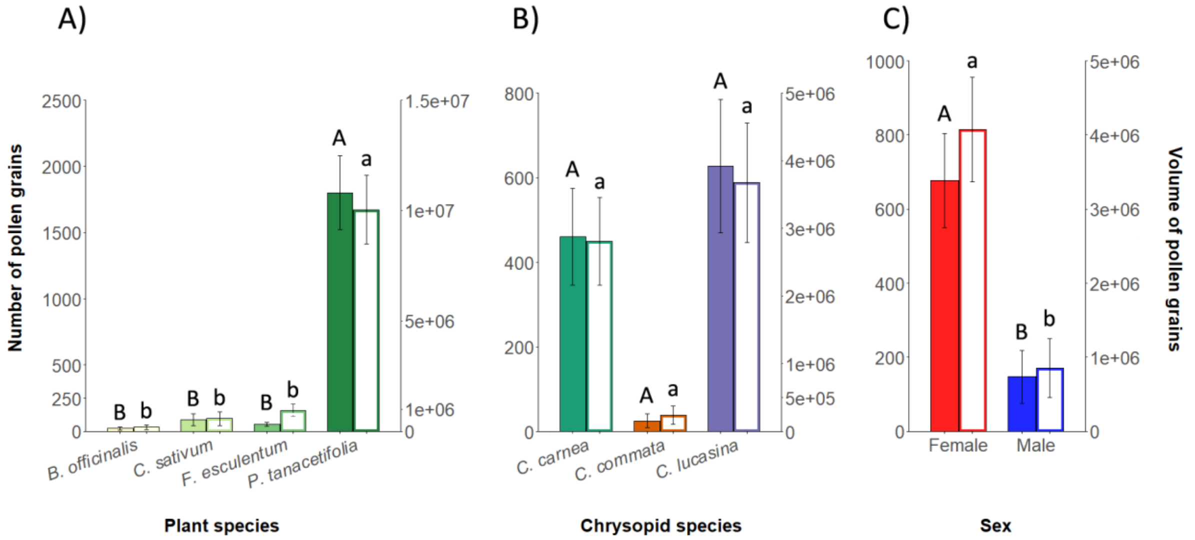

Figure 8. Number (left axes) and volume (right axes) of pollen grains (mean ± SE) consumed by the chrysopids collected from the flower strips by A) plant species, B) chrysopid species and C) sex. Solid bars represent the number of pollen grains and hollow bars represent the volume of pollen grains. Significant differences are indicated with upper case letters for number of pollen grains and lower case letters for volume of pollen grains (GLMM, Turkey's tests, $P < 0.05$).

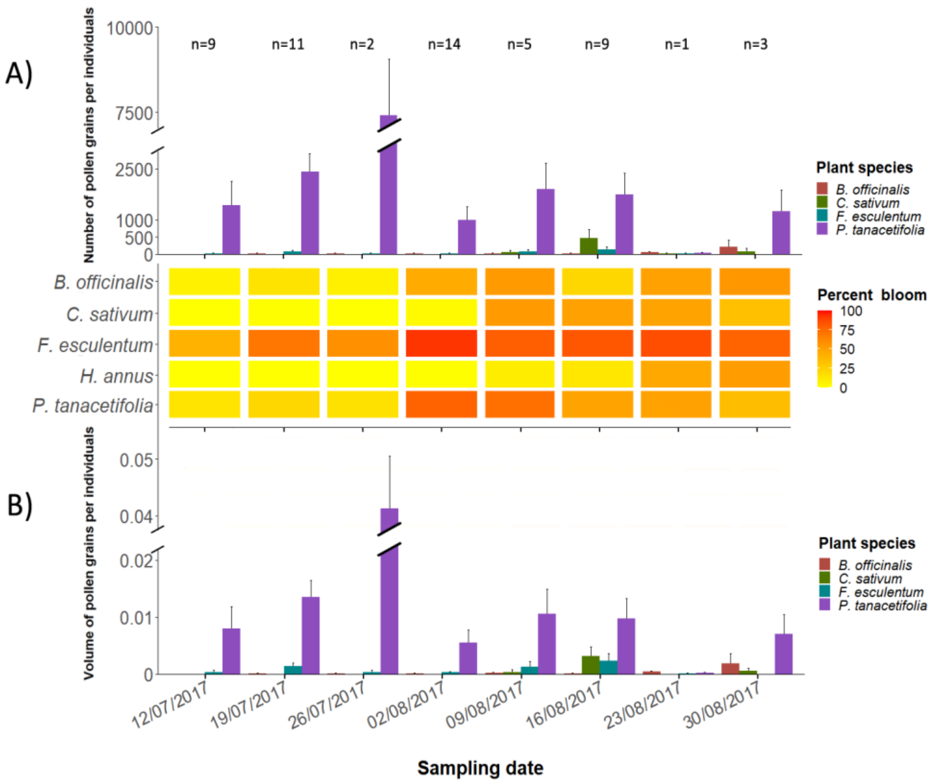

Figure 9. A) Number and B) volume of pollen grains (mean ± SE) consumed by the chrysopids collected (n) from the flower strips in each sampling date. The heatmap represents the percent bloom of each plant species in the flower strips.

4. Discussion

The implementation of our tailored flower strips in Brassica crops enhanced the community of arthropods, thus increasing the functional biodiversity in the studied agro-ecosystem.

Our findings of visual inspections in the crop showed an impact on *B. oleracea* pests in the vicinity of flower strips compared to locally reduced biocontrol in the control blocks, while we found a similar abundance of natural enemies in flower strips and control blocks. We could not find a synchronization between predators; the five studied Brassica pests and the flower bloom. Although, it could indicate that predators may have stayed locally in the flower strips with access to all the resources required, such as shelter, mating, pollen, nectar or alternative prey.

Interestingly, we observed a high attraction of predators and parasitoids to the flower strips. Parasitoids were highly correlated with the flower bloom, which indicates the high dependence of plant resources of this group. Predator abundance seemed to be driven by other factors not considered in this study, however, the farm location played an important role on predator community. For instance, in Farm 2 compared to the other two farms, the highest density of surrounding trees might have influenced the higher abundance of predators detected.

The Chrysopidae community recorded in the flower strips was composed by three species, two of them, *C. carnea sensu* Henry and *C. lucasina*, very frequently found in crops and commonly associated with herbaceous vegetation. From their pollen feeding preferences, we observed that *P. tanacetifolia* was the plant species most consumed and preferred by Chrysopidae, as observed in previous studies (Villenave et. al 2013). One of the most interesting results observed is that Chrysopidae females consumed a higher number of pollen grains compared to males. This has already been observed by other authors and explained based on the needs of females for floral resources for reproduction (Villenave et. al 2005, Alcalá Herrera et. al 2020).

5. Conclusion

In conclusion, flower strips may have a positive effect on biological pest control in Brassica crops. Indeed, the use of flower strips may be an alternative to pesticide applications, creating a more favourable environment for locally occurring natural enemies that have the potential to control key brassica pests. *P. tanacetifolia* is pointed out as a particularly suitable plant to increase the presence of *C. carnea* females in agricultural fields. Enhancing natural enemy biodiversity using flower resources, as observed for green lacewings, can increase the resilience of natural pest control and provide additional ecosystem services such as pollination. As a general recommendation, we encourage researchers, farmers and practitioners to include the establishment of flower strips in their integrated pest management programs as a promising tool to protect functional biodiversity for biocontrol.

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